APPENDIX B

Benthic Community Assessment

CONTENTS

RAPID BIOASSESSMENT PROTOCOL: BENTHIC MACROINVERTEBRATES (EPA 1989, 1999)

As with the habitat assessments, there are more advanced and complex methods for characterizing benthic communities than what is presented below. However, the Rapid Bioassessment Protocols (RBP) outlined by the U.S. EPA (EPA 1989, 1999) have been proven to be efficient and effective in small streams and rivers. The EPA is currently developing guidance for benthic characterization in lakes, large rivers, and coastal areas. States such as Ohio, Maine, and North Carolina use approaches that are also very useful, and similar in many ways. The following are direct excerpts from EPA (1989, 1999; www.epa.gov/owow/monitoring/rbp) and Ohio EPA (1989) guidance manuals. For more extensive information, the reader should refer directly to those manuals. In addition to the references given in the following text, other useful information for identifying benthic macroinvertebrates is found in Barbour et al. 1999; Beck 1977; Harris and Lawrence 1978; Hubbard and Peters 1978; Surdick and Gaufin 1978; USDA 1985.

Rapid Bioassessment Protocol (RBP) utilizes the systematic field collection and analysis of major benthic taxa. The data are compiled into various metrics. The optimal metrics will vary across (and even within) ecoregions, so a qualified benthic ecologist should be used to select the most appropriate metrics. The protocol can be used to prioritize sites for more intensive evaluation (i.e., replicate sampling, ambient toxicity testing, chemical characterization). The EPA 1989 guid-

ance described three levels of RBPs, each with more accurate taxonomic resolution. This approach also recommended sampling a single habitat type. The 1999 guidance describes methods for multi-habitat assessments, which are more appropriate in low-gradient streams and rivers where there is little cobble and riffle area. The description below focuses on single habitat characterization.

Sample Collection

The collection procedure provides representative samples of the macroinvertebrate fauna from comparable habitat types at all stations constituting a site evaluation, and is supplemented with separate coarse particulate organic matter (CPOM) samples (e.g., leaves, decaying vegetation). This RBP single habitat approach focuses on the riffle/run habitat because it is the most productive habitat available in stream systems and includes many pollution-sensitive taxa of the scraper and filtering collector functional feeding groups. The CPOM sample provides a measure of effects (particularly toxicity effects) on a third trophic component of the benthic community, the shredders.

In sampling situations where a riffle/run habitat with a rock substrate is not available, any submerged fixed structure will provide a substrate for the scraper and filtering collector functional groups emphasized here. This allows for the same approach to be used in non-wadable streams and large rivers and wadable streams and rivers with unstable substrates.

Riffle/Run Sample

Riffle areas with relatively fast currents and cobble and gravel substrates generally provide the most diverse community. Riffles should be sampled using a kick net to collect from an approximately 1-m^2 area. A minimum of two 1-m^2 riffle samples should be collected at each station: one from an area of fast current and one from an area of slower current. The samples are composited for processing. In streams lacking riffles, run areas with cobble or gravel substrate are also appropriate for kick net sampling.

Where riffle/run communities with a rock substrate are not available, other submerged fixed structures (e.g., submerged boulders, logs, bridge abutments, pier pilings) should be sampled by hand picking. These structures provide suitable habitat for the scrapers and filtering collectors and will allow use of the RBP in a wider range of regions and stream orders.

CPOM Sample

In addition to the riffle/run sample collected for evaluation of the scraper and filtering collector functional feeding groups, a CPOM sample should also be collected to provide data on the abundance of shredders at the site. Large particulate shredders are important in forested areas of stream ecosystems ranging from stream orders 1 through 4 (Minshall et al. 1985). The absence of shredders of large particulate material is characteristic of unstable, poorly retentive headwater streams in disturbed watersheds or in dry areas where leaf material processing is accomplished by terrestrial detritivores (Minshall et al. 1985). McArthur et al. (1988) reported that very few shredders were found in summer leaf packs in South Carolina because processing was so rapid.

The CPOM sample is processed separately from the riffle/run sample and used only for characterizing the functional feeding group representation. Sampling the CPOM component requires a composite collection of various plant parts such as leaves, needles, twigs, bark, or their fragments. Potential sample sources include leaf packs, shore zones, and other depositional areas where CPOM may accumulate. Only the upper surface of litter accumulation in depositional areas should be sampled to ensure that it is from the aerobic zone. For the shredder community analysis, several handfuls of material should be adequate. A variety of CPOM forms should be collected if available. CPOM collected may be washed in a dip net or a sieve bucket.

Shredder abundance is maximum when the CPOM is partially decomposed (Cummins et al. 1989). Care must be taken to *avoid* collecting recent or fully decomposed leaf litter to optimize collection of the shredder community. For this CPOM collection technique, seasonality may have an important influence on shredder abundance data. For instance, fast-processing litter (e.g., basswood, alder, maples, birch) would have the highest shredder representation in the winter (Cummins et al. 1989). The slow-processing litter (e.g., oaks, rhododendrons, beech, conifers) would have the highest shredder representation in the summer.

Sample Sorting and Identification

Riffle/Run Sample

Sorting and enumeration in the field to obtain a 100 (or higher) -count organism subsample is recommended for the riffle/run sample. After processing in the field, the organisms and sample residue should be preserved for archiving. Thus, a reanalysis (for quality control) or more thorough processing (e.g., larger counts, more detailed taxonomy) would be possible. The subsampling method described in this protocol is based on Hilsenhoff's Improved Biotic Index (Hilsenhoff 1987) and is similar to that used by the New York Department of Environmental Conservation (Bode 1988). This subsampling technique provides for a consistent unit of effort and a representative estimate of the benthic fauna (modified from Hilsenhoff 1987):

1. Thoroughly rinse sample in a (500-μm) screen or the sampling net to remove fine sediments. Any large organic material (whole leaves, twigs, algal or macrophyte mats) should be rinsed, visually inspected, and discarded.
2. Place sample contents in a large, flat pan with a light-colored (preferably white) bottom. The bottom of the pan should be marked with a numbered grid pattern, each block in the grid measuring 5×5 cm. (Sorting using a gridded pan is only feasible if the organism movement in the sample can be slowed by the addition of club soda or tobacco to the sample. If the organisms are not anesthetized, 100 organisms should be removed from the pan as randomly as possible.) A 30×45 cm pan is generally adequate, although pan size ultimately depends on sample size. Larger pans allow debris to be spread more thinly, but they are unwieldy. Samples too large to be effectively sorted in a single pan may be thoroughly mixed in a container with some water, and half of the homogenized sample placed in each of two gridded pans. Each half of the sample must be composed of the same kinds and quantity of debris, and an equal number of grids must be sorted from each pan to ensure a representative subsample.
3. Add just enough water to allow complete dispersion of the sample within the pan; excessive water will allow sample material to shift within the grid during sorting. Distribute sample material evenly within the grid.
4. Use a random numbers table to select a number corresponding to a square within the gridded pan. Remove all organisms from within that square and proceed with the process of selecting squares and removing organisms until the total number sorted from the sample is within 10% of 100. Any organism that is lying over a line separating two squares is considered to be in the square containing its head. In those instances where it is not possible to determine the location of the head (worms for instance), the organism is considered to be in the square containing the largest portion of its body. Any square sorted must be sorted in its entirety, even after the 100 count has been reached. In order to lessen sampling bias, the investigator should attempt to pick smaller, cryptic organisms as well as the larger, more obvious ones.

An alternative method of subsampling live samples in the field is to simply sort 100 organisms in a random manner. Narcotization to slow the organisms is less important with this subsampling technique. To lessen sampling bias, the investigator should pick smaller, cryptic organisms, as well as the larger, more obvious organisms.

BENTHIC MACROINVERTEBRATE FIELD DATA SHEET

STREAM NAME	LOCATION	
STATION #_____ RIVERMILE_____	STREAM CLASS	
LAT _____ LONG _____	RIVER BASIN	
STORET #	AGENCY	
INVESTIGATORS		LOT NUMBER
FORM COMPLETED BY	DATE _____ TIME _____ AM PM	REASON FOR SURVEY

HABITAT TYPES	**Indicate the percentage of each habitat type present** ❑ Cobble_____% ❑ Snags_____% ❑ Vegetated Banks_____% ❑ Sand_____% ❑ Submerged Macrophytes_____% ❑ Other (_____)_____%
SAMPLE COLLECTION	**Gear used** ❑ D-frame ❑ kick-net ❑ Other _____ **How were the samples collected?** ❑ wading ❑ from bank ❑ from boat **Indicate the number of jabs/kicks taken in each habitat type.** ❑ Cobble_____ ❑ Snags_____ ❑ Vegetated Banks_____ ❑ Sand_____ ❑ Submerged Macrophytes_____ ❑ Other (_____)_____
GENERAL COMMENTS	

QUALITATIVE LISTING OF AQUATIC BIOTA
Indicate estimated abundance: 0 = Absent/Not Observed, 1 = Rare, 2 = Common, 3= Abundant, 4 = Dominant

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

FIELD OBSERVATIONS OF MACROBENTHOS
Indicate estimated abundance: 0 = Absent/Not Observed, 1 = Rare (1-3 organisms), 2 = Common (3-9 organisms), 3= Abundant (>10 organisms), 4 = Dominant (>50 organisms)

Porifera	0	1	2	3	4	Anisoptera	0	1	2	3	4	Chironomidae	0	1	2	3	4
Hydrozoa	0	1	2	3	4	Zygoptera	0	1	2	3	4	Ephemeroptera	0	1	2	3	4
Platyhelminthes	0	1	2	3	4	Hemiptera	0	1	2	3	4	Trichoptera	0	1	2	3	4
Turbellaria	0	1	2	3	4	Coleoptera	0	1	2	3	4	Other	0	1	2	3	4
Hirudinea	0	1	2	3	4	Lepidoptera	0	1	2	3	4						
Oligochaeta	0	1	2	3	4	Sialidae	0	1	2	3	4						
Isopoda	0	1	2	3	4	Corydalidae	0	1	2	3	4						
Amphipoda	0	1	2	3	4	Tipulidae	0	1	2	3	4						
Decapoda	0	1	2	3	4	Empididae	0	1	2	3	4						
Gastropoda	0	1	2	3	4	Simuliidae	0	1	2	3	4						
Bivalvia	0	1	2	3	4	Tabinidae	0	1	2	3	4						
						Culcidae	0	1	2	3	4						

Figure B.1 Benthic macroinvertebrate field data sheet. (From EPA. *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 841/B-99/002. 1999.)

All organisms in the subsample should be classified according to functional feeding group. Field classification is important because many families comprise genera and species representing a variety of functional groups. Knowing the family-level identification of the organisms will generally be insufficient for categorization by functional feeding group. Functional feeding group classification can be done in the field, on the basis of morphological and behavioral features, using Cummins and Wilzbach (1985). Care should be taken in noting early instars, which may constitute different functional feeding groups from the later instars. Recommended forms for recording benthic data are presented in Figures B.1 through B.4 (EPA 1999).

The scraper and filtering collector functional groups are the most important indicators in the riffle/run community. Numbers of individuals representing each of these two groups are recorded on the Benthic Macroinvertebrate Field Data Sheet (Figure B.1) (EPA 1999). The Benthic

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BENTHIC MACROINVERTEBRATE SAMPLE LOG-IN SHEET

Date Collected	Collected By	Number of Containers	Preservation	Station #	Stream Name and Location	Date Received by Lab	Lot Number	Date of Completion		
								sorting	mounting	identification

Serial Code Example: B0754001(1)
B = Benthos (F = Fish; P = Periphyton) ■ 0754 = project number ■ 001 = sample number ■ (1) = lot number (e.g., winter 1996 =1; summer 1996 = 2)

Figure B.2 Benthic macroinvertebrate sample log-in sheet. (From EPA. *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 841/B-99/002. 1999.)

Macroinvertebrate Sample Log-In Sheet (Figure B.2) (EPA 1999) is used to record all collections and is an important part of the QA/QC and sample tracking activities.

All organisms in the subsample should be identified to family or order, enumerated, and recorded, along with any observations on abundance of other aquatic biota, on this data sheet. A summary of all benthic data to be used in the final analysis will be recorded on the Benthic Macroinvertebrate Laboratory Bench Sheet (Figures B.3 and B.4) (EPA 1999) upon return to the laboratory. The use of family-level identification in this protocol is based on Hilsenhoff's Family Biotic Index, which uses higher taxonomic levels of identification (Hilsenhoff 1988).

CPOM Sample

Organisms collected in the supplemental CPOM sample are classified as shredders or non-shredders. Taxonomic identification is not necessary for this component. The composited CPOM sample may be field sorted in a small pan with a light-colored bottom or in the net or sieve through which it was rinsed. (If a large number of benthic macroinvertebrates have been collected, a representative subsampling of 20 to 60 organisms may be removed for functional feeding group classification.) Numbers of individuals representing the shredder functional group, as well as total number of macroinvertebrates collected in this sample, should be recorded for later analysis. The shredder/nonshredder metric may be deemed optional in rivers or in some regions where shredder abundance is naturally low. However, the potential utility of such a metric for assessing toxicant effects warrants serious consideration in this bioassessment approach.

Data Analysis Techniques

Biological impairment of the benthic community may be indicated by the absence of generally pollution-sensitive macroinvertebrate taxa such as Ephemeroptera, Plecoptera, and Trichoptera (EPT); excess dominance by any particular taxon, especially pollutant-tolerant forms such as some

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (FRONT)

page _____ of _____

STREAM NAME	LOCATION
STATION #_____ RIVERMILE_____	STREAM CLASS
LAT _____ LONG _____	RIVER BASIN
STORET #	AGENCY
COLLECTED BY DATE_____	LOT #
TAXONOMIST DATE_____	SUBSAMPLE TARGET ❑ 100 ❑ 200 ❑ 300 ❑ Other ____

Enter Family and/or Genus and Species name on blank line.

Organisms		No.	LS	TI	TCR	Organisms		No.	LS	TI	TCR
Oligochaeta						Megaloptera					
Hirudinea						Coleoptera					
Isopoda											
Amphipoda						Diptera					
Decapoda											
Ephemeroptera											
						Gastropoda					
						Pelecypoda					
Plecoptera											
						Other					
Trichoptera											
Hemiptera											

Taxonomic certainty rating (TCR) 1-5:1=most certain, 5=least certain. If rating is 3-5, give reason (e.g., missing gills). LS= life stage: I = immature; P = pupa; A = adult TI = Taxonomists initials

Total No. Organisms _____ **Total No. Taxa** _____

Figure B.3 Benthic macroinvertebrate laboratory bench sheet (front). (From EPA. *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 841/B-99/002. 1999.)

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (BACK)

SUBSAMPLING/SORTING INFORMATION Sorter _____ Date _____	Number of grids picked: _____ Time expenditure _____ No. of organisms _____ Indicate the presence of large or obviously abundant organisms: **QC:** ❑ YES ❑ NO **QC Checker** _____ # organisms originally sorted ÷ (# organisms recovered by checker + # organisms originally sorted) = % sorting efficiency ≥90%, sample passes _____ <90%, sample fails, action taken _____
TAXONOMY ID _____ Date _____	Explain TCR ratings of 3-5: Other Comments (e.g. condition of specimens): **QC:** ❑ YES ❑ NO **QC Checker** _____ Organism recognition ❑ pass ❑ fail Verification complete ❑ YES ❑ NO

General Comments (use this space to add additional comments):

Figure B.4 Benthic macroinvertebrate laboratory bench sheet (back). (From EPA. *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 841/B-99/002. 1999.)

Chironomidae and Oligochaeta taxa; low overall taxa richness; or appreciable shifts in community composition relative to the reference condition. Impairment may also be indicated by an overabundance of fungal slimes or filamentous algae, or an absence of expected populations of fish. All of these indicators can be evaluated using the sampling data generated. A number of useful metrics exist (Tables B.2 and B.3), while Figure B.5 (EPA 1999) is a preliminary assessment score sheet.

On the basis of observations made in the assessment of habitat, water quality, physical characteristics, and the qualitative biosurvey, the investigator concludes whether impairment is detected. If impairment is detected, an estimation of the probable cause and source should be made. The aquatic biota that indicated an impairment, are noted along with observed indications of potential

PRELIMINARY ASSESSMENT SCORE SHEET
(PASS)

page ____ of ____

STREAM NAME	LOCATION
STATION # _____ RIVERMILE _____	STREAM CLASS
LAT _____ LONG _____	RIVER BASIN
STORET #	AGENCY
COLLECTED BY DATE _____	LOT # _____ NUMBER OF SWEEPS _____
HABITATS: ❑ COBBLE ❑ SHOREZONE ❑ SNAGS ❑ VEGETATION	

Enter Family and/or Genus and Species name on blank line.

Organisms	No.	LS	TI	TCR	Organisms	No.	LS	TI	TCR
Oligochaeta					Megaloptera				
Hirudinea					Coleoptera				
Isopoda									
Amphipoda					Diptera				
Decapoda									
Ephemeroptera					Gastropoda				
					Pelecypoda				
Plecoptera					Other				
Trichoptera									
Hemiptera					Taxonomic certainty rating (TCR) 1-5:1=most certain, 5=least certain. If rating is 3-5, give reason (e.g., missing gills). LS= life stage: I = immature; P = pupa; A = adult TI = Taxonomists initials				

	Site Value	Target Threshold	If 2 or more metrics are ≥ target threshold, site is
Total No. Taxa			**HEALTHY**
EPT Taxa			If less than 2 metrics are within target range, site is
Tolerance Index			**SUSPECTED IMPAIRED**

Figure B.5 Preliminary assessment score sheet. (From EPA. *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 841/B-99/002. 1999.)

problem sources. The downstream extent of impact is estimated and multiplied by appropriate stream width to provide an estimate of the areal extent of the problem.

The data analysis scheme used in this RBP integrates several community, population, and functional parameters into a single evaluation of biotic integrity. Each parameter, or metric, measures a different component of community structure and has a different range of sensitivity to pollution stress (Figure B.6). This integrated approach provides more assurance of a valid assessment because a variety of parameters are evaluated. Deficiency of any one metric in a particular situation should not invalidate the entire approach.

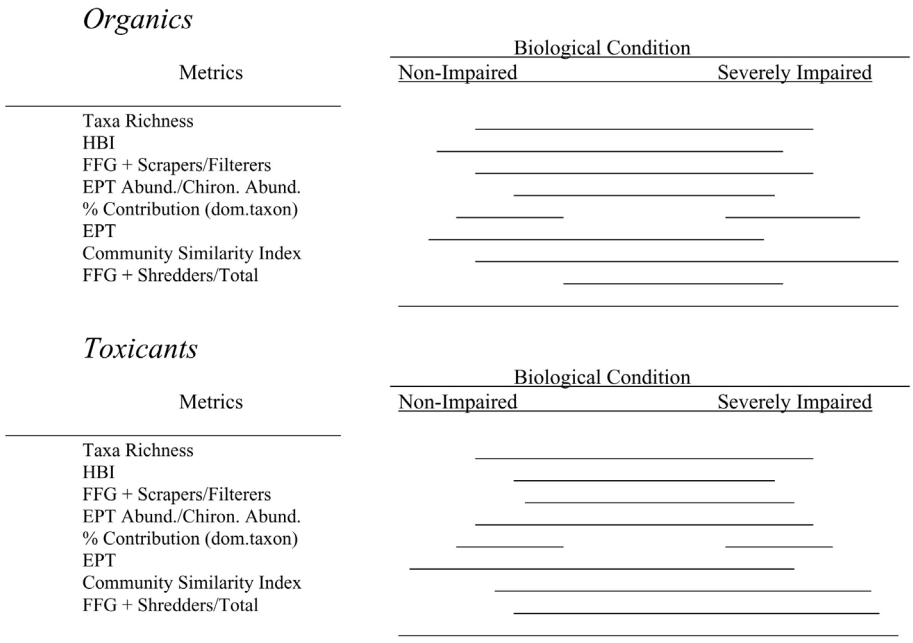

Figure B.6 Range of sensitivities of Rapid Bioassessment Protocol II and III benthic metrics in assessing biological condition in response to organics and toxicants.

The integrated data analysis (Figure B.7) is performed as follows. Using the raw benthic data, a numerical value is calculated for each metric. Calculated values are then compared to values derived from either a reference site within the same region, a reference database applicable to the region, or a suitable control station on the same stream. Each metric is then assigned a score according to the comparability (percent similarity) of calculated and reference values. Scores for the eight metrics are then totaled and compared to the total metric score for the reference station. The percent comparison between the total scores provides a final evaluation of biological condition. The criteria to be used for scoring the eight metrics *may need to be adjusted for use in particular regions.*

Inherent variability in each metric was considered in establishing percent comparability criteria (Figure B.6). The metrics based on taxa richness, FBI, and EPT Indices have low variability (Resh 1988). This variability is accounted for in the criteria for characterization of biological condition, based on existing data. For metrics based on standard taxa richness and FBI and EPT Indices, differences of 10 to 20% relative to the reference condition would be considered nominal, and the station being assessed would receive the maximum metric score. Because increasing FBI values denote worsening biological condition, percent difference for this metric is calculated by dividing the reference value by the value for the station of comparison.

Metrics that utilize ratios fluctuate more widely, however, and comparing percent differences between ratios (ratios of ratios) will compound the variability. Scoring increments are therefore set at broad intervals of 25% or greater. For metrics based on functional feeding group ratios, Cummins (1987, personal communication) contends that differences as great as 50% from the reference may be acceptable, but differences in the range of 50 to 100% are not only important, but discriminate degrees of impact more clearly.

The contribution of the dominant taxon to total abundance is a simple estimator of evenness. Scoring criteria are based on theoretical considerations rather than direct comparison with a reference.

The Community Loss Index (a representative similarity index) already incorporates a comparison with a reference. Therefore, actual index values are used in scoring.

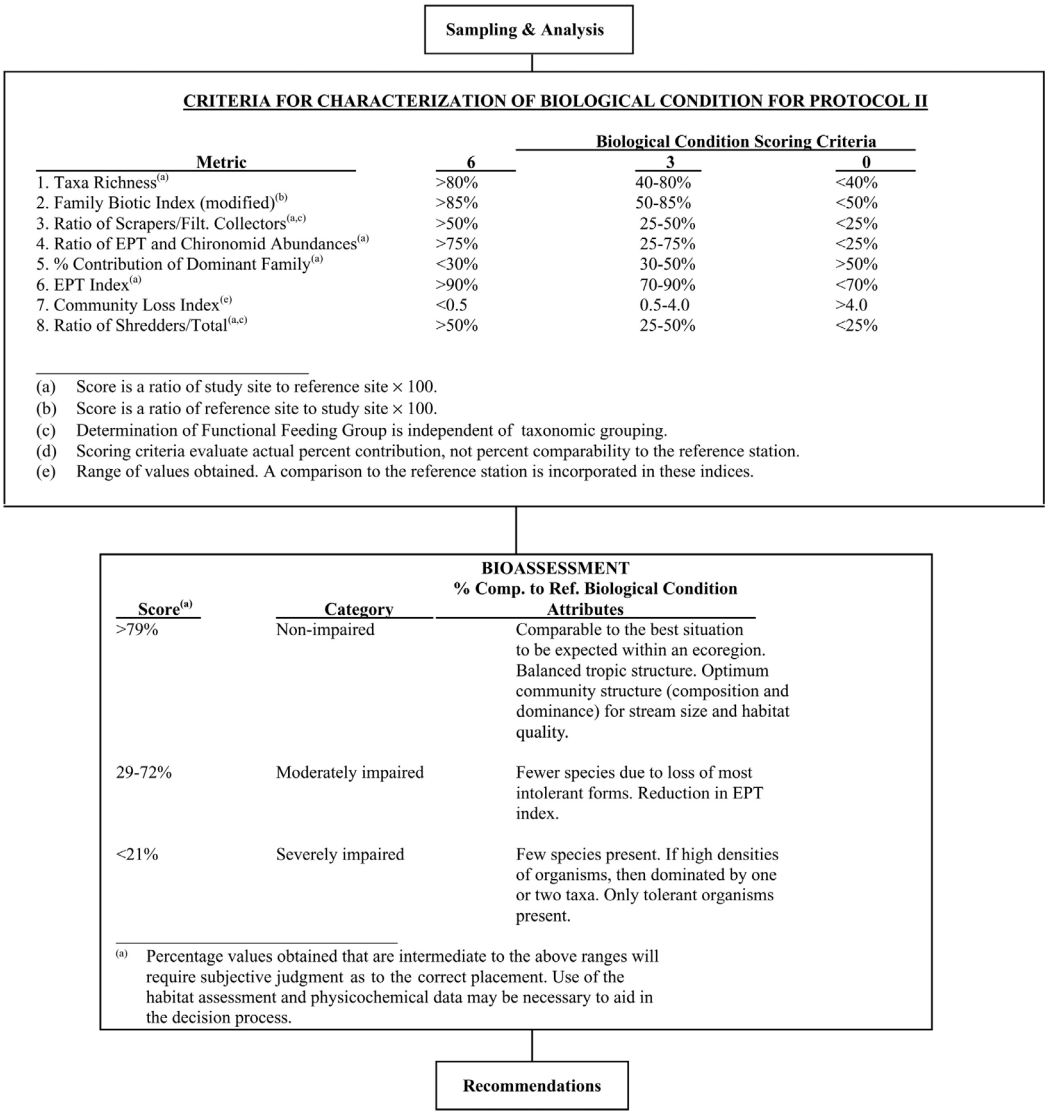

Figure B.7 Flowchart of bioassessment approach advocated for a Rapid Bioassessment Protocol.

The metrics used to evaluate the benthic data and their significance are explained below and in Tables B.1 and B.2.

Riffle/Run Sample

Metric 1. Taxa Richness

Reflects health of the community through a measurement of the variety of taxa (total number of families) present. Generally increases with increasing water quality, habitat diversity, and habitat suitability. Sampling of highly similar habitats will reduce the variability in this metric attributable to factors such as current speed and substrate type. Some pristine headwater streams may be naturally unproductive, supporting only a very limited number of taxa. In these situations, organic enrichment may result in an increased number of taxa (including EPT taxa).

Table B.1 Definitions of Best Candidate Benthic Metrics and Predicted Direction of Metric Response to Increasing Perturbation

Category	Metric	Definition	Predicted Response to Increasing Perturbation
Richness measures	Total No. taxa	Measures the overall variety of the macroinvertebrate assemblage	Decrease
	No. EPT taxa	Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)	Decrease
	No. Ephemeroptera taxa	Number of mayfly taxa (usually genus or species level)	Decrease
	No. Plecoptera taxa	Number of stonefly taxa (usually genus of species level)	Decrease
	No. Trichoptera taxa	Number of caddisfly taxa (usually genus or species level)	Decrease
Composition measures	% EPT	Percent of the composite of mayfly, stonefly, and caddisfly larvae	Decrease
	% Ephemeroptera	Percent of mayfly nymphs	Decrease
Tolerance/intolerance measures	No. intolerant taxa	Taxa richness of those organisms considered to be sensitive to perturbation	Decrease
	% tolerant organisms	Percent of macrobenthos considered to be tolerant of various types of perturbation	Increase
	% dominant taxon	Measures the dominance of the single most abundant taxon. Can be calculated as dominant 2, 3, 4, or 5 taxa.	Increase
Feeding measures	% filterers	Percent of the macrobenthos that filter FPOM from either the water column or sediment	Variable
	% grazers and scrapers	Percent of the macrobenthos that scrape or graze upon periphyton	Decrease
Habit measures	No. clinger taxa	Number of taxa of insects	Decrease
	% clingers	Percent of insects having fixed retreats or adaptations for attachment to surfaces in flowing water.	Decrease

Data from DeShon 1995; Barbour et al. 1996b; Fore et al. 1996; Smith and Voshell 1997.

Metric 2. Modified Family Biotic Index

Tolerance values range from 0 to 10 for families and increase as water quality decreases. The index was developed by Hilsenhoff (1988) to summarize the various tolerances of the benthic arthropod community with a single value. The Modified Family Biotic Index was developed to detect organic pollution and is based on the original species-level index (Hilsenhoff 1982). Tolerance values for each family were developed by weighting species according to their relative abundance in the State of Wisconsin.

The family-level index has been modified for this document to include organisms other than just arthropods using the genus and species-level biotic index developed by the State of New York (Bode 1988). The formula for calculating the Family Biotic Index is:

$$HBI = \frac{\sum x_i t_j}{n}$$

where x_i = number of individuals within a taxon
t_j = tolerance value of a taxon
n = total number of organisms in the sample

Table B.2 Definitions of Additional Potential Benthic Metrics and Predicted Direction of Metric Response to Increasing Perturbation

Category	Metric	Definition	Predicted Response to Increasing Perturbation	References
Richness measures	No. *Pteronarcys* species	The presence or absence of a long-lived stonefly genus (2–3 year life cycle)	Decrease	Fore et al. 1996
	No. Diptera taxa	Number of "true" fly taxa, which includes midges	Decrease	DeShon 1995
	No. Chironomidae taxa	Number of taxa of chironomid (midge) larvae	Decrease	Hayslip 1993; Barbour et al. 1996b
Composition measures	% Plecoptera	Percent of stonefly nymphs	Decrease	Barbour et al. 1994
	% Trichoptera	Percent of caddisfly larvae	Decrease	DeShon 1995
	% Diptera	Percent of all "true" fly larvae	Increase	Barbour et al. 1996b
	% Chironomidae	Percent of midge larvae	Increase	Barbour et al. 1994
	% Tribe Tanytarsini	Percent of Tanytarisinid midges to total fauna	Decrease	DeShon 1995
	% Other Diptera and noninsects	Composite of those organisms generally considered to be tolerant to a wide range of environmental conditions	Increase	DeShon 1995
	% *Corbicula*	Percent of Asiatic clam in the benthic assemblage	Increase	Kerans and Karr 1994
	% Oligochaeta	Percent of aquatic worms	Variable	Kerans and Karr 1994
Tolerance/ intolerance measures	No. intol. snail and mussel species	Number of species of molluscs generally thought to be pollution intolerant	Decrease	Kerans and Karr 1994
	% sediment tolerant organisms	Percent of infaunal macrobenthos tolerant of perturbation	Increase	Fore et al. 1996
	Hilsenhoff Biotic Index	Uses tolerance values to weight abundance in an estimate of overall pollution; originally designed to evaluate organic pollution	Increase	Barbour et al. 1992; Hayslip 1993; Kerans and Karr 1994
	Florida Index	Weighted sum of intolerant taxa, which are classed as 1 (least tolerant) or 2 (intolerant); Florida Index = 2 × Class 1 taxa + Class 2 taxa	Decrease	Barbour et al. 1996b
	% Hydropsychidae to Trichoptera	Relative abundance of pollution tolerant caddisflies (metric could also be regarded as a composition measure)	Increase	Barbour et al. 1992; Hayslip 1993
Feeding measures	% omnivores and scavengers	Percent of generalists in feeding strategies	Increase	Kerans and Karr 1994
	% ind. gatherers and filterers	Percent of collector feeders of CPOM and FPOM	Variable	
	% gatherers	Percent of the macrobenthos that "gather"	Variable	Barbour et al. 1996b
	% predators	Percent of the predator functional feeding group; can be made restrictive to exclude omnivores	Variable	Kerans and Karr 1994
	% shredders	Percent of the macrobenthos that "shreds" leaf litter	Decrease	Barbour et al. 1992; Hayslip 1993
Life cycle measures	% multivoltine	Percent of organisms having short (several per year) life cycle	Increase	Barbour et al. 1994
	% univoltine	Percent of organisms relatively long-lived (life cycles of 1 or more years)	Decrease	Barbour et al. 1994

Hilsenhoff's family-level tolerance values may require modification for some regions. Alternative tolerance classifications and biotic indices have been developed by some state agencies. Additional biotic indices are listed in EPA (1983).

Although the FBI may be applicable for toxic pollutants, it has only been evaluated for organic pollutants. The State of Wisconsin is conducting a study to evaluate the ability of Hilsenhoff's index to detect nonorganic effects.

Metric 3. Ratio of Scraper and Filtering Collector Functional Feeding Groups

The scraper and filtering collector metric reflects the riffle/run community foodbase. When compared to a reference site, shifts in the dominance of a particular feeding type indicate a community responding to an overabundance of a particular food source. The predominant feeding strategy reflects the type of impact detected. Assignment of individuals to functional feeding groups is independent of taxonomy, with some families representing several functional groups.

A description of the functional feeding group concept can be found in Cummins (1973) and Merritt and Cummins (1984). Functional feeding group designations for most aquatic insect families may be found in Merritt and Cummins (1984). Most aquatic insects can also be classified to functional feeding group in the field, on the basis of morphological and behavioral features, using Cummins and Wilzbach (1985).

The relative abundance of scrapers and filtering collectors in the riffle/run habitat is an indication of the periphyton community composition, availability of suspended fine particulate organic material (FPOM), and availability of attachment sites for filtering. Scrapers increase with increased diatom abundance and decrease as filamentous algae and aquatic mosses (which scrapers cannot efficiently harvest) increase. However, filamentous algae and aquatic mosses provide good attachment sites for filtering collectors, and the organic enrichment often responsible for overabundance of filamentous algae can also provide FPOM that is utilized by the filterers.

Filtering collectors are also sensitive to toxicants bound to fine particles and should be the first group to decrease when exposed to steady sources of such bound toxicants. This situation is often associated with point-source discharges where certain toxicants adsorb readily to dissolved organic matter (DOM), forming FPOM during flocculation. Toxicants thus become available to filterers via FPOM. The scraper to filtering collector ratio may not be a good indicator of organic enrichment if adsorbing toxicants are present. In these instances the FBI and EPT Index may provide additional insight. Qualitative field observations on periphyton abundance may also be helpful in interpreting results.

Metric 4. Ratio of EPT and Chironomidae Abundances

The EPT and Chironomidae abundance ratio uses relative abundance of these indicator groups (Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae) as a measure of community balance. Good biotic condition is reflected in communities with an even distribution among all four major groups and with substantial representation in the sensitive groups Ephemeroptera, Plecoptera, and Trichoptera. Skewed populations having a disproportionate number of the Chironomidae relative to the more sensitive insect groups may indicate environmental stress (Ferrington 1987; Shackleford 1988). Certain species of some genera such as *Cricotopus* are highly tolerant (Lenat 1983; Mount et al. 1984), and as opportunists may become numerically dominant in habitats exposed to metal discharges where EPT taxa are not abundant, thereby providing a good indicator of toxicant stress (Winner et al. 1980). Clements et al. (1988) found that mayflies were more sensitive than chironomids to exposure levels of 15 to 32:g/L of copper. Chironomids tend to become increasingly dominant in terms of percent taxonomic composition and relative abundance along a gradient of increasing enrichment or heavy metals concentration (Ferrington 1987).

An alternative to the ratio of EPT and Chironomidae abundance metric is the Indicator Assemblage Index (IAI) developed by Shackleford (1988). The IAI integrates the relative abundances of the EPT taxonomic groups and the relative abundances of chironomids and annelids upstream and downstream of a pollutant source to evaluate impairment. The IAI may be a valuable metric in areas where the annelid community may fluctuate substantially in response to pollutant stress.

Metric 5. Percent Contribution of Dominant Family

The percent contribution of the dominant family to the total number of organisms uses abundance of the numerically dominant taxon relative to the rest of the population as an indication of community balance at the family level. A community dominated by relatively few families would indicate environmental stress. This metric may be redundant if the Pinkham and Pearson Similarity Index is used as a community similarity index for metric number 7.

Metric 6. EPT Index

The EPT Index generally increases with increasing water quality. The EPT Index value is the total number of distinct taxa within the groups Ephemeroptera, Plecoptera, and Trichoptera. The EPT Index value summarizes the taxa richness within the insect groups that are generally considered pollution sensitive. This was developed for species-level identifications; however, the concept is valid for use at family-level identifications.

Headwater streams which are naturally unproductive may experience an increase in taxa (including EPT taxa) in response to organic enrichment.

Metric 7. Community Similarity Indices

Community Similarity Indices are used in situations where a reference community exists, either through sampling or through prediction for a region. Data sources or ecological data files may be available to predict a reference community to be used for comparison. The combined information provided through a regional analysis and EPA's ERAPT ecological database (Dawson and Hellenthal 1986) may be useful for this analysis. These indices are designed to be used with either species level identifications or higher taxonomic levels. Three of the many community similarity indices available are discussed below:

- Community Loss Index. Measures the loss of benthic taxa between a reference station and the station of comparison. The Community Loss Index was developed by Courtemanch and Davies (1987) and is an index of compositional dissimilarity, with values increasing as the degree of dissimilarity with the reference station increases. Values range from 0 to "infinity." Based on preliminary data analysis, this index provides greater discrimination than either of the following two community similarity indices.
- Jaccard Coefficient of Community Similarity. Measures the degree of similarity in taxonomic composition between two stations in terms of taxon presence or absence. The Jaccard Coefficient discriminates between highly similar collections. Coefficient values, ranging from 0 to 1.0, increase as the degree of similarity with the reference station increases. See Jaccard (1912), Boesch (1977), and EPA (1983) for more detail. The formulae for the Community Loss Index and the Jaccard Coefficient are

$$\text{Community Loss} = \frac{d - a}{e}$$

$$\text{Jaccard Coefficient} = \frac{a}{a + b + c}$$

where a = number of taxa common to both samples
b = number of taxa present in Sample B but not A
c = number of taxa present in Sample A but not B
d = total number of taxa present in Sample A
e = total number of taxa present in Sample B
Sample A = reference station (or mean of reference database)
Sample B = station of comparison

- Pinkham and Pearson Community Similarity Index Incorporates abundance and compositional information and can be calculated with either percentages or numbers. A weighting factor can be added that assigns more significance to dominant taxa. See Pinkham and Pearson (1976) and EPA (1983) for more detail. The formula is

$$S.I._{ab} = \sum \frac{\min(x_{ia}, x_{ib})}{\max(x_{ia}, x_{ib})} \left[\frac{\frac{x_{ia}}{x_a} \bullet \frac{x_{ib}}{x_b}}{2} \right]$$

where x_{ia}, x_{ib} = number of individuals in the ith taxon in Sample A or B

Other community similarity indices include Spearman's Rank Correlation (Snedecor and Cochran 1967), Morisita's Index (Morisita 1959), Biotic Condition Index (Winget and Mangum 1979), and Bray-Curtis Index (Bray and Curtis 1957; Whittaker 1952). Calculation of a chi-square "goodness of fit" (Cochran 1952) may also be appropriate.

CPOM Sample

Metric 8. Ratio of Shredder Functional Feeding Group and Total Number of Individuals Collected

Also based on the Functional Feeding Group concept, the abundance of the shredder functional group relative to the abundance of all other functional groups allows evaluation of potential impairment as indicated by the CPOM-based shredder community. Shredders are sensitive to riparian zone impacts and are particularly good indicators of toxic effects when the toxicants involved are readily adsorbed to the CPOM and either affect microbial communities colonizing the CPOM or the shredders directly (Cummins 1987, personal communication).

The degree of toxicant effects on shredders vs. filterers depends on the nature of the toxicants and the organic particle adsorption efficiency. Generally, as the size of the particle decreases, the adsorption efficiency increases as a function of the increased surface to volume ratio (Hargrove 1972). Because waterborne toxicants are readily adsorbed to FPOM, toxicants of a terrestrial source (e.g., pesticides, herbicides) accumulate on CPOM prior to leaf fall, thus having a substantial effect on shredders (Swift et al. 1988a,b). The focus on this approach is on a comparison to the reference community which should have a reasonable representation of shredders as dictated by seasonality, region, and climate. This allows for an examination of shredder or collector "relative" abundance as indicators of toxicity.

The data collected in the 100-organism riffle/run subsample and the CPOM sample are summarized according to the information required for each metric and entered on the Data Summary Sheet.

Each metric result is given a score based on percent comparability to a reference station. Scores are totaled and compared to the total metric score for the reference station. The percent comparison between the total scores provides a final evaluation of biological condition. Values obtained may sometimes be intermediate to established ranges and require some judgment as to assessment of

biological condition. In these instances, habitat assessment, physical characterization, and water quality data may aid in the evaluation process.

Guidance for Data Summary Sheets for Benthic RBP

Station Number: Indicate station number for each data set recorded.

Station Location: Record brief description of sampling site relative to established landmarks (i.e., roads, bridges).

Taxa Richness: Record total number of families (or higher taxa) collected in the 100-organism riffle subsample.

FBI (modified): Record the Family Biotic Index value (Hilsenhoff 1988) calculated for the 100-organism riffle subsample using the formula presented in RBP II. Tolerance classification values can be entered into the computer database to simplify calculation.

Functional Feeding Group: Functional feeding group classifications may be entered into the computer database to simplify calculations.

Riffle Community: Scrapers/filtering collectors: enter the value obtained by dividing the number of individuals in the riffle subsample representing the scraper functional group, by the number representing the filtering collector functional group.

CPOM Community: Shredders/total: enter the value obtained by dividing the number of individuals in the CPOM sample (or subsample) representing the shredder functional group, by the total number of organisms in the sample (or subsample).

EPT/Chironomidae: Enter the value obtained by dividing the number of individuals in the 100-organism riffle subsample in the family Chironomidae, by the total number of individuals in the orders Ephemeroptera, Plecoptera, and Trichoptera.

Percent Contribution (Dominant Family): Record the value obtained by dividing the number of individuals in the family that is most abundant in the 100-organism riffle subsample, by the total number of individuals in the sample.

EPT Index: Record the total number of taxa in the 100-organism riffle subsample representing the orders Ephemeroptera, Plecoptera, and Trichoptera.

Community Similarity Index: Enter the value calculated for the appropriate community similarity index, using data from the 100-organism riffle subsample.

Values obtained for each metric should be assigned a score based on percent comparability to the control or reference station data. Scores are summed for both the impaired and reference station. The percent comparison between the total scores provides the final evaluation of biological condition.

Family-Level Tolerance Classification

The original RBP II (EPA 1989) is based on family-level identifications. The adequate assessment of biological condition for RBP II requires the use of a tolerance classification for differentiating among responses of the benthic community to pollutants. Hilsenhoff's Family Biotic Index (FBI) is used as a basis for the family-level tolerance classification.

The biotic index (BI) of organic pollution is adapted (Hilsenhoff 1987) for rapid evaluation by providing tolerance values for families (Tables B.3 and B.4) to allow a family-level biotic index (FBI) to be calculated in the field. The FBI is an average of tolerance values of all arthropod families in a sample. It is not intended as a replacement for the BI and can be effectively used in the field only by biologists who are familiar enough with arthropods to be able to identify families without using keys.

Using the same method and more than 2000 stream samples from throughout Wisconsin that were used to revise tolerance values for species and genera (Hilsenhoff 1987) family-level tolerance values were established by comparing occurrence of each family with the average BI of streams in which they occurred in the greatest numbers. Thus, family-level tolerance values tend to be a weighted average of tolerance values of species and genera within each family based on their relative abundance in Wisconsin.

Table B.3 Tolerance Values for Families of Stream Arthropods in the Western Great Lakes Region

Plecoptera	Capniidae 1, Chloroperlidae 1, Leuctridae 0, Nemouridae 2, Perlidae 1, Perlodidae 2, Pteronarcyidae 0, Taeniopterygidae 2
Ephemeroptera	Baetidae 4, Baetiscidae 3, Caenidae 7, Ephemerellidae 1, Ephemeridae 4, Heptageniidae 4, Leptophlebiidae 2, Metretopodidae 2, Oligoneuriidae 2, Polymitarcyidae 2, Potomanthidae 4, Siphlonuridae 7, Tricorythidae 4
Odonata	Aeshnidae 3, Calopterygidae 5, Coenagrionidae 9, Cordulegastridae 3, Corduliidae 5, Gomphidae 1, Lestidae 9, Libellulidae 9, Macromiidae 3
Trichoptera	Brachycentridae 1, Glossosomatidae 0, Helicopsychidae 3, Hydropsychidae 4, Hydroptilidae 4, Lepidostomatidae 1, Leptoceridae 4, Limnephilidae 4, Molannidae 6, Odontoceridae 0, Philopotamidae 3, Phryganeidae 4, Polycentropodidae 6, Psychomyiidae 2, Rhyacophilidae 0, Sericostomatidae 3
Megaloptera	Corydalidae 0, Sialidae 4
Lepidoptera	Pyralidae 5
Coleoptera	Dryopidae 5, Elmidae 4, Psephenidae 4
Diptera	Athericidae 2, Blephariceridae 0, Ceratopogonidae 6, Blood-red Chironomidae (Chironomini) 8, other (including pink) Chironomidae 6, Dolochopodidae 4, Empididae 6, Ephyridae 6, Psychodidae 10, Simuliidae 6, Muscidae 6, Syrphidae 10, Tabanidae 6, Tipulidae 3
Amphipoda	Gammaridae 4, Talitridae 8
Isopoda	Asellidae 8

Data from Hilsenhoff, W.L. Rapid field assessment of organic pollution with a family-level biotic index. *J. North Am. Benthol. Soc.*, 7: 65–68. 1988; EPA. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 444/4-89/001. 1989.

THE OHIO EPA INVERTEBRATE COMMUNITY INDEX APPROACH (OEPA 1989)

Field Methods — Quantitative Sampling

The primary sampling equipment used for the collection of benthic macroinvertebrates is the modified Hester–Dendy multiple-plate artificial substrate sampler. The sampler is constructed of $^1/_8$-in tempered hardboard cut into 3-in square (or circular) plates and 1-in square spaces. A total of eight plates and 12 spacers are used for each sampler. The plates and spacers are placed on a $^1/_4$-in stainless steel eyebolt so that there are three single spaces, three double spaces, and one triple space between the plates. The total surface area of the sampler, excluding the eyebolt, is 145.6 in^2.

Samplers placed in streams are tied to a concrete construction block, which anchors them in place and prevents the multiple-plates from coming into contact with the natural substrates. In water deeper than 4 ft, a float (1 quart cubitainer) is attached to the samplers to keep them within 4 ft of the surface. Whenever possible, the samplers are placed in runs rather than pools or riffles and an attempt is made to establish stations in as similar an ecological situation as possible. All samplers are exposed for a 6-week period. A set of samplers consists of three multiple-plate samplers (about 3 ft^2 of surface area) at National Ambient Water Quality Monitoring Network (NAWQMN) stations and five multiple-plate samplers at all other sampling locations. All NAWQMN stations and most routine monitoring stations are sampled from June 15 to September 30.

Retrieval of the sampler is accomplished by cutting them from the block and placing them in 1-quart, wide-mouth plastic containers while still submersed. Care is taken to avoid disturbing the samplers and thereby dislodging any organisms. Enough formalin is added to each container to equal an approximate 10% solution.

Qualitative samples of macroinvertebrates inhabiting the natural substrates are also collected at the time of sampler retrieval. In shallow water, samples are taken in a stream segment covering all available habitats near where the samplers were placed. Samples are collected using triangular ring frame 30-mesh dip nets and hand picking with forceps. Grab samplers (i.e., Ekman, Peterson, or Ponar) can also be used in deep water. The qualitative sampling continues until, by gross examination, no new taxa are being taken. A station description sheet is filled out by the collector at the time

Table B.4 . Tolerance Values for Some Macroinvertebrates Not Included in Hilsenhoff (1982, 1987)

Acariformes	4
Decapoda	6
Gastropoda	
Amnicola	8
Bithynia	8
Ferrissia	6
Gyraulus	8
Helisoma	6
Lymnaea	6
Physa	8
Sphaeriidae	8
Oligochaeta	
Chaetogaster	6
Dero	10
Nais barbata	8
Nais behningi	6
Nais bretscheri	6
Nais communis	8
Nais elinguis	10
Nais pardalis	8
Nais simplex	6
Nais variabilis	10
Pristina	8
Stylaria	8
Tubificidae	
Aulodrilus	8
Limnodrilus	10
Hirudinea	
Helobdella	10
Turbellaria	4

From Bode, R.W. *Quality Assurance Workplan for Biological Stream Monitoring in New York State*. New York State Department of Environmental Conservation, Albany. 1988; EPA. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 444/4-89/001. 1989.

of sampler retrieval. The substrate is described using the categories for substrate characterization indicated in the U.S. EPA biological field manual (Weber 1973).

In situations where quantitative biological samples are collected from the natural substrates using a Surber square foot sampler (30-mesh netting), the collector stands on the downstream side of the sampler and works the substrate using a hand cultivator with 2-in tines. Large rocks are gently scrubbed with a brush. The material collected is placed in sealed containers, preserved in 10% formalin, and transported to the laboratory. Three to five Surber samples are taken at each site.

In situations where Ekman, Peterson, or Ponar grab samples are used for quantitative purposes, three to five samples are collected and then treated in essentially the same manner as the Surber samples. The material collected with the grab is washed through a bucket with a 30-mesh screen bottom, placed in sealed containers, preserved in 10% formalin, and returned to the laboratory.

Laboratory Methods — Quantitative Sampling

Samples are coded and sample numbers are immediately entered into a log book upon arrival at the laboratory. Samples are given a log number derived from the date, e.g., 871108-10, where 87 represents the year, 11 represents the month, and 08 the day. The number following this six-digit date, i.e., the number 10 in the previous example, indicates that this was the 10th sample logged that day. Other information in the log book includes the name(s) of field personnel who collected the sample, date, stream or lake name, basin name, entity (where applicable), general location, sample type, sampling method(s) used, the person who conducted the analyses, and any other comments considered pertinent to the collection and analysis of the sample.

Macroinvertebrate Counts and Identifications

Composite samples consisting of five multiple-plate samplers are used in station evaluations for routine monitoring. However, replicate samples (three multiple-plate samplers) are reported to the EPA for NAWQMN stations. Replicate sets of five multiple-plate samplers can be used if deemed necessary in cases where sampling is for litigation purposes. In all cases, the multiple-plate samplers are disassembled in a bucket of water and cleaned of organisms and debris. The organism/debris mixture is then passed through U.S. Standard Testing Sieves number 30 (0.589-mm openings) and number 40 (0.425-mm openings). The material retained in each sieve is preserved in properly labeled and coded jars containing 70% alcohol.

The following procedures are used during the course of analyzing an artificial substrate, Surber, or grab sample:

1. Sorting the sample is done in a white enamel pan followed by scanning under the dissecting microscope (10× magnification). Subsamples are produced using the following guidelines:
   a. A Folsom sample splitter is used for all subsampling. In an effort to determine the accuracy of the Folsom sample splitter, a sample composed of 200 individuals of five frequently collected organisms was prepared and repeatedly split. Statistical analysis of the data yielded a chi-square value of 2.56, df = 4, indicating that the subsamples were not significant at the 95% probability level.
   b. After an entire sample has been sorted, subsampling within families containing unmanageable numbers is acceptable.
   c. Very large samples may be subsampled prior to sorting, but only after examination in a white enamel pan to remove obvious rare taxa, e.g., hellgramites, non-hydropsychid caddisflies.
   d. A minimum of 250 organisms are identified, with at least 50 to 100 midges, 70 caddisflies, 70 mayflies.
2. Dipterans of the family Chironomidae are prepared for identification by clearing the larvae in hot 10% KOH for 30 min and then mounting in water on microscope slides. Permanent slides for the voucher collection are mounted in Euparol mounting medium.
3. Material retained in the #40 screen is counted and identified or counted and extrapolated when identification is impossible or impractical. (Artificial substrate sample only.)
4. Organisms determined to be dead before the time of collection are discarded.
5. When only one sex or life stage can be identified, it is assumed that the other sex or stage is the same species.
6. Sections of bryozoan colonies are removed from the plates and saved for identification. Only colonies, not individuals, are counted. (Artificial substrate sample only.)
7. Early instars that cannot be identified are extrapolated where possible.
8. Species-level identifications are made where possible and practical. Generic or higher level classifications are made if specimens are damaged beyond identification, in those cases where taxonomy is incomplete or laborious and time-consuming, or where the specimen is an unidentifiable early instar.
9. Organisms are listed in tables following the laboratory table format.
10. Two end fragments of an oligochaete are counted as one individual. Fragments without ends are not counted.
11. Any taxonomic key in the laboratory may be used as an aid in the identification of an organism. Also indicated is the level of taxonomy attainable with the keys listed.

Macroinvertebrate Data Analysis

Invertebrate Community Index

The principal measure of overall macroinvertebrate community condition used by the Biological Field Evaluations Group is the Invertebrate Community Index (ICI), a measurement derived in-house from information collected over many years. The ICI is a modification of the Index of Biotic Integrity (IBI) for fish developed by Karr (1981). The ICI consists of 10 structural community metrics, each with four scoring categories of 6, 4, 2, and 0 points (Table B.5). The point system evaluates a sample against a database of 247 relatively undisturbed reference sites throughout Ohio. Six points will be scored if a given metric has a value comparable to those of exceptional stream communities, 4 points for those metric values characteristic of more typical good communities, 2 points for metric values slightly deviating from the expected range of good values, and 0 points for metric values strongly deviating from the expected range of good values. The summation of the individual metric scores (determined by the relevant attributes of an invertebrate sample with some consideration given to stream drainage area) results in the ICI value. Metrics 1 through 9 are all generated from the artificial substrate sample data, while Metric 10 is based solely on the

Table B.5 . Invertebrate Community Index (ICI) Metrics and Scoring Criteria Based on Macroinvertebrate Community Data from 247 Reference Sites throughout Ohio

Metric		Scoring Criteria			
		0	2	4	6
1.	Total number of taxa	Scoring of each metric varies with drainage area; see Ohio EPA (1987)			
2.	Total number of mayfly taxa				
3.	Total number of caddisfly taxa				
4.	Total number of dipteran taxa				
5.	Percent mayflies				
6.	Percent caddisflies				
7.	Percent tribe tanytarsini midges				
8.	Percent other dipterans and non-insects				
9.	Percent tolerant organisms				
10.	Total number of qualitative Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa				

qualitative sample data from natural substrates. More discussion of the derivation of the ICI including descriptions of each metric and the data plots and other information used to score each metric can be found in Ohio EPA (1987).

Community Similarity Index

A coefficient of similarity between two stations can be calculated using Van Horn's (1950) equation modified from the general formula described by Gleason (1920):

$$c = \frac{2w}{a + b}$$

The variables in this expression can be based either on the number of taxa present or absent at each station or on actual numerical data collected at each site. If the presence/absence method is being used:

a = the number of taxa collected at one station
b = the number of taxa collected at the other station
w = the number of taxa common to both stations

When actual numerical data are being used, each taxon is assigned a prominence value calculated by multiplying the density of the taxon by the square root of its frequency of occurrence (Beals 1961; Burlington 1962). In this case:

a = the sum of the prominence values of all of the taxa at one station
b = the sum of the prominence values of all of the taxa at the other station
c = the sum of the prominence values of all of the taxa of one station which it has in common with the other station. The lower of the two resulting values of w is used in the equation.

Rank Correlation Coefficient

A rank correlation coefficient between measured biological, chemical, or other physical data can be calculated using the formula defined by Spearman (1904):

$$r_s = 1 - \frac{6\sum_{i-1}^{n} D_i^2}{n(n^2 - 1)}$$

where n = the number of paired observations ($x_i y_i$) and D_i = the rank of x_i minus the rank of y_i.

Table B.6 Benthic Macroinvertebrate Equipment and Supplies

Item	Unit	Source[a]
Boat, flat bottom, 14–16 ft, snatch-block meter, wheel and trailer, 18 hp outboard motor. Life jackets, other accessories	1	(7,15)
Boat crane kit and winch	1	(3,15)
Boat, inflatable with oar set	1	(1,15)
Cable fastening tools		(4,15)
Cable clamps, 1/8"	25	
Nicro-press clamps, 1/8"	100	
Nicro-press tool, 1/8"	1	
Wire cutter, Felco	1	
Wire thimbles, 1/8"	25	
Cable, 1/8", galvanized steel	1000 ft	(3,15)
Large capacity metal wash tub	1	
Sample wash bucket (sieve)	1	(8,14)
Core sampler, hand held	1	(3,8,14)
Box corer	1	(14)
K-B corer	1	(8)
Wide-barrel gravity corer	1	(14)
Phleger corer	1	(8,14)
Ballchek single or multiple corer	1	(8,14)
Ewing portable piston corer	1	(14)
Hardboard multiplate sampler	10	(3,8)
Ceramic multiplate sampler	10	(14)
Trawl net	1	(8)
Dredge	1	(3,8,14)
Rectangular box sediment sampler	1	(14)
Drift net, stream	6	(8,14)
Triple-net drift sampler	2	(14)
Stream bottom sampler, Surber type	2	(3,8,14)
Portable invertebrate box sampler	2	(13)
Stream-bed fauna sampler, Hess type	2	(14)
Hess stream bottom sampler	2	(8)
Grab sampler, Ponar	1	(3,8,14)
Wildco box corer	1	(8)
Grab sampler, Ekman	1	(3,8,14)
Grab sampler, Petersen	1	(3,8,14)
Grab sampler, Smith-McIntyre	1	(14)
Grab sampler, Van Veen	1	(14)
Grab sampler, Orange Peel	1	(14)
Grab sediment sampler, Shipek	1	(8)
Basket, bar B-Q, tumbler (#740-0035)	12	(9,11)
Sieves, US Standard No. 30	2	(5)
Flowmeter, mechanical	1	(3)
Mounting media, CMCP-9/9AF with stain	4 oz	No longer available
Mounting medium, CMCP-9	4 oz	(6)
Mounting medium, CMCP-10	4 oz	(6)
Fuchsin basic, C.I. dye	25 g	(6)
Mounting medium, Aquamount	4 oz	(12)
Refrigerated circulator	1	(5)
Water pump, epoxy-coated	2	(1)
Holding tank, constant temp	1	(10)
Balance, top-loading	1	(5)
Counter, 12-unit, 2×6	1	(3)
Counter, hand tally	2	(3)
Waders, with suspenders	1 pr	(1,15)
Boots, hip	1 pr	(1,15)
Raincoat	1	(3,15)

Table B.6 Benthic Macroinvertebrate Equipment and Supplies (continued)

Item	Unit	Source[a]
Magni-focuser, 2×	1	(5)
Microscope, field	1	(3)
Magnifier, illuminated + base	1	(3)
Magnifier, pocket, 5×, 10×, and 15×	1	(3)
Microscope, compound, with phase and bright-field, trinocular, 10× and 15× eyepieces, 4×, 10×, 20×, 45× and 100× objectives	1	(5)
Microscope, stereoscopic, with stand	1	(2)
Microscope slide dispenser	1	(1)
Microscope slides and cover slips, 12 and 15 mm circles	10 gross	(1)
Photographic system, photostar	1	(5)
Camera, photomicrographic, with 50 mm lens	1	(1,15)
Stirrer, magnetic	1	(5)
Aquarium, 10 gal., with cover, air pump and filter	1	(1,15)
Aquatic dip net, Model 412D	2	(3)
Jars, screw cap, specimen	5 dz	(1)
Bottles, wide mouth, 32 oz	1 case	(1)
Specimen jars, wide mouth, 4 oz	48	(1)
Specimen jars, wide mouth, 6 oz	48	(1)
Vials, specimen, 1 oz	10 gross	(1)
Petri dish, ruled grid	4	(1)
Petri dish, compartmented	1 case	(1)
Watch glasses	10	(1)
Vacuum oven	1	(5)
Sounding lead and calibrated line	1	(3)
Forceps, watchman's, stainless	1 pr	(1)
Forceps, microdissection	2 pr	(1)
Dissecting set, basic	1	(1)
Water test kit, limnology	1	(1)
Thermometer, digital	1	(1)
Wash bottle, wide mouth, 500 mL	4	(1)
Wash bottle, polyethylene, 4 oz	2	(1)
Dropper bottle, polystop, 30 mL	2	(2)
Desiccator, polypropylene	1	(1)
Clipboard with cover	2	(3,15)
Calculator, scientific	1	(3,15)
Marker, permanent, black	2	(3,15)
Pen set, slim pack, Koh-i-noor	1	(3,15)
Heavy paper tags with string	1000	(1,15)
Ice chest, insulated, 48 qt	2	(3,15)
Blue ice, soft pack	10	(3,15)
Plastic bags	100	(3,15)
Formalin, 10%	4 L	(2)
Ethyl alcohol	20 L	(2)
Trays, polypropylene, sorting	6	(5)

Note: Listed above are equipment and supplies needed for the collection and analysis of macroinvertebrate samples. The data quality objectives and sampling and analysis methods should determine the type of equipment and supplies needed. The source numbers refer to the companies that are listed at the end of the table. Mention of these sources or products does not constitute endorsement by the U.S. Environmental Protection Agency.

Table B.6 Benthic Macroinvertebrate Equipment and Supplies (continued)

[a] Sources of equipment and supplies:

1. Carolina Biological Supply Co.
   2700 York Road
   Burlington, NC 27215
2. Fisher Scientific
   50 Fadem Road
   Springfield, NJ 07081
3. Forestry Suppliers, Inc.
   205 West Rankin Street
   Jackson, MS 39284-8397
4. Industrial Rope Supply
   5250 River Road
   Cincinnati, OH 45233
5. Curtin Matheson Scientific, Inc.
   9999 Veterans Memorial Drive
   Houston, TX 77038-2499
6. Polyscience
   400 Valley Road
   Warrington, PA 18976
7. MonArk Boat Company
   Monticello, AK 71655
8. Wildlife Supply Company
   301 Case Street
   Saginaw, MI 48602
9. Tenaco
   2007 NE 27th Avenue
   Gainesville, FL 32609
10. Frigid Units, Inc.
    3214 Sylvania Avenue
    Toledo, OH 43613
11. W.C. Bradley Enterprises, Inc.
    P.O. Box 1240
    Columbus, GA 31993
12. Gallard-Schlesinger Chemical Mfg. Corp.
    584 Mineola Avenue
    Carle Place, NY 11514
13. Ellis-Rutter Associates
    P.O. Box 401
    Punta Gorda, FL 33950
14. Kahl Scientific Instrument Corp.
    P.O. Box 1166
    El Cajon, CA 92022-1166
15. Locally

From EPA. *Biological Criteria: Guide to Technical Literature*. U.S. Environmental Protection Agency, Washington, D.C. EPA-440-5-91-004. 1991.

Coefficient of Variation

In cases where replicate analyses are conducted (e.g., litigation purposes of NAWQMN stations), a coefficient of variation (CV or COV) between replicates is determined following the procedures outlined by Li (1964) using the formula:

$$CV = \frac{s}{x} \bullet 100\%$$

where s = the sample standard deviation
x = the sample mean.

A PARTIAL LISTING OF AGENCIES THAT HAVE DEVELOPED TOLERANCE CLASSIFICATIONS AND/OR BIOTIC INDICES

Florida Department of Environmental Regulation
Illinois EPA
New York Department of Environmental Conservation
North Carolina Department of Environmental Management
Ohio EPA
U.S. Department of Agriculture, Forest Service, Intermountain Region
U.S. EPA Region V
Vermont Department of Environmental Conservation

Table B.7. Phylogenetic Order for Macroinvertebrate Listing Including Level of Taxonomy Generally Used

Porifera:	Species	Plecoptera	
Coelenterata:	Genus	Pteronarcyidae:	Genus
Platyhelminthes:	Class	Peltoperfidae:	Genus
Nematomorpha:	Genus	Taeniopterygidae:	Genus
Bryozoa:	Species	Nemounidae:	Species
Entoprocta:	Species	Leuctridae:	Genus
Annelida		Capniidae:	Genus
Oligochaeta:	Class	Perfidae:	Species
Hirudinea:	Species	Perlodidae:	Species
Arthropoda		Chloroperfidae:	Genus
Crustacea		Hemiptera	
Isopoda:	Genus	Belostomatidae:	Genus
Amphipoda:	Genus/Species	Nepidae:	Genus
Decapoda:	Species	Pleidae:	Genus
Arachnoidea		Naucoridae:	Genus
Hydracarina:	Class	Corixidae:	Genus
Insecta		Notonectidae:	Genus
Ephemeroptera		Megaloptera	
Siphlonuridae:	Genus	Sialidae:	Genus
Baetidae:	Genus	Corydalidae:	Species
Oligoneuriidae:	Genus	Neuroptera:	Genus
Heptageniidae:	Genus/Species	Trichoptera	
Leptophlebiidae	Genus	Philopotamidae:	Genus/Species
Ephemerelidae:	Species	Psychomyiidae:	Species
Tricorythidae:	Genus	Polycentropodidae:	Genus
Caenidae:	Genus	Hydropsychidae:	Genus/Species
Baetiscidae:	Species	Rhyacophilidae:	Genus/Species
Potamanthidae:	Genus	Glossosomatidae:	Genus
Ephemeridae:	Genus	Hydroptidae:	Genus/Species
Polymitarchidae:	Species	Phryganeidae:	Genus
Odonata		Brachycentridae:	Genus
Zygoptera		Limnophilidae:	Genus
Calopterygidae:	Genus	Lepidostomatidae:	Genus
Lestidae:	Species	Beraeidae:	Genus
Coenagrionidae:	Family/Genus	Sericostomatidae:	Genus
Anisoptera		Odontocaridae:	Genus
Aeshnidae:	Species	Molannidae:	Genus
Gomphidae:	Species	Helicopsychidae:	Species
Cordulegastridae:	Species	Calamoceratidae:	Genus
Macromiidae:	Species	Leptocaridae:	Genus/Species
Corduliidae:	Species	Lepidoptera:	Genus
Libellulidae:	Species		

Table B.8. Level of Macroinvertebrate Taxonomy Attainable Using Keys

Coleoptera	
Gynnidae:	Genus
Haliplidae:	Genus
Dytiscidae:	Genus
Noteridae:	Genus
Hydrophilidae:	Genus
Hydraenidae:	Genus
Psepheriidae:	Species
Dryopidae:	Genus
Scirtidae:	Family
Elmidae:	Genus/Species
Limnichidae:	Genus
Heteroceridae:	Family
Ptilodactylidae:	Family
Chrysomelidae:	Family
Curculionidae:	Family
Lampyridae:	Family
Diptera	
Tipulidae:	Genus
Psychodidae:	Genus
Ptychopteridae:	Genus
Dixidae:	Genus
Chaoboridae:	Genus
Culicidae:	Genus
Thaumaleidae:	Genus
Simuliidae:	Genus
Certopogonidae:	Family/Genus/Species
Chironomidae	
Tanypodinae:	Genus/Species
Diamesinae:	Genus/Species
Prodiamesinae:	Genus/Species
Orthocladinae:	Genus/Species
Chironominae	
Chironomini:	Genus/Species
Pseudochironomini:	Genus/Species
Tanytarsini:	Genus/Species
Tabanidae:	Genus/Species
Athericidae:	Species
Stratiomyidae:	Genus
Empididae:	Family
Dolichopodidae:	Family
Syrphidae:	Family/Genus
Sciomyzidae:	Family/Genus
Ephydridae:	Family/Genus
Muscidae:	Species
Mollusca	
Gastropoda:	Family/Genus/Species
Pelecypoda:	Family/Genus/Species

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